08:00:36 OCA PAD AMENDMENT - PROJECT HEADER INFORMATION 05/12/93

Active

Project #: G-33-A17 Cost share #: Rev #: 1
Center # : 10/24-6-Q5250-7A0 Center shr #: OCA file #:
Work type : RES
Contract#: 5 R01 EY01746-17 Mod #: RNOA OF 4/15/93 Document : GRANT
Prime #: Contract entity: GTRC

Subprojects ? : N CFDA: 93.868
Main project #: PE #: N/A

Project unit: CHEMISTRY Unit code: 02.010.136
Project director(s):
YU N-T CHEMISTRY (404)894-4007

Sponsor/division names: DHHS/PHS/NIH / NATL INSTITUTES OF HEALTH
Sponsor/division codes: 108 / 001

Award period: 920501 to 940430 (performance) 940730 (reports)

Sponsor amount	New this change	Total to date
Contract value	0.00	253,296.00
Funded	0.00	253,296.00
Cost sharing amount		0.00

Does subcontracting plan apply ?: N

Title: COMPARATIVE RAMAN STUDIES OF HUMAN AND ANIMAL LENSES

PROJECT ADMINISTRATION DATA

OCA contact: Kathleen R. Ehlinger 894-4820

Sponsor technical contact

DR. LORE ANN MCNICOL
(000)000-0000

EXTRAMURAL PROGRAM DIRECTOR
CATARACT PROGRAM
NATIONAL EYE INSTITUTE
9000 ROCKVILLE PIKE
BETHESDA, MD

Sponsor issuing office

MARIA ROOS
(301)496-5301

GRANTS MANAGEMENT OFFICE
EXTRAMURAL SERVICES BRANCH
NATIONAL EYE INSTITUTE
9000 ROCKVILLE PIKE
BETHESDA, MD.

Security class (U,C,S,TS) : U ONR resident rep. is ACO (Y/N): N
Defense priority rating : N/A NIH supplemental sheet
Equipment title vests with: Sponsor GIT X

Administrative comments -
ISSUED TO EXTEND TERMINATION DATE FROM 4/30/93 TO 4/30/94.

GEORGIA INSTITUTE OF TECHNOLOGY
OFFICE OF CONTRACT ADMINISTRATION

NOTICE OF PROJECT CLOSEOUT

Closeout Notice Date 11/02/94

Project No. G-33-A17_____ Center No. 10/24-6-Q5250-7A0_

Project Director YU N-T_____ School/Lab CHEMISTRY_____

Sponsor DHHS/PHS/NIH/NATL INSTITUTES OF HEALTH_____

Contract/Grant No. 5 R01 EY01746-17_____ Contract Entity GTRC

Prime Contract No. _____

Title COMPARATIVE RAMAN STUDIES OF HUMAN AND ANIMAL LENSES_____

Effective Completion Date 940430 (Performance) 940730 (Reports)

Closeout Actions Required:	Y/N	Date Submitted
Final Invoice or Copy of Final Invoice	Y	_____
Final Report of Inventions and/or Subcontracts	Y	_____
Government Property Inventory & Related Certificate	N	_____
Classified Material Certificate	N	_____
Release and Assignment	N	_____
Other _____	N	_____

Comments_____
NOTE USE DHHS FORM FOR PATENT._____

Subproject Under Main Project No. _____

Continues Project No. G-33-A17_____

Distribution Required:

Project Director	Y
Administrative Network Representative	Y
GTRI Accounting/Grants and Contracts	Y
Procurement/Supply Services	Y
Research Property Managment	Y
Research Security Services	N
Reports Coordinator (OCA)	Y
GTRC	Y
Project File	Y
Other _____	N
_____	N

NOTE: Final Patent Questionnaire sent to PDPI.

G-33-A17
2

Final Progress Report
of
NIH project: 5 R01 EY01746-17

Title of the Project: Comparative Raman Studies of Human and Animal Lenses

Period covered : May 1, 1988 - April 30, 1994.

Institution: Georgia Institute of Technology

The personel who have worked on the project:

Yu, Nai-Teng	P.I.
Kuck, J. F. R., Jr.	Research Collaborator / Prof. of Ophthalmology at Emory Univ.
Nie, S.	Postdoctoral Res. Associate / Res. Scientist; Now Assistant Prof. at Indiana Univ.
Lo, W.-K.	Research Collaborator / Prof. of Anatomy at Morehouse Univ.
Su, Kai C.	Research Collaborator / Executive Vice-President of CIBA-Vision
Bergbauer, K. L.	Graduate Student, completed her Ph.D. degree
Zigman, S.	Research Collaborator / Prof. of Ophthalmology at Univ. of Rochester
Lou, M. F.	Res. Collaborator / Alcon Labs.
Chen, Wenlung	Graduate Student, completed his Ph.D. degree
Horwitz, J.	Res. Collaborator / Prof. of Ophthalmology at UCLA
Cai, M. Z.	Research Associate
Lee, B. S.	Postdoctoral Research Associate
Yu, S.-C.	Postdoctoral Research Associate
Bursell, S.-E.	Research Collaborator / Joslin Diabetes Center
Castillo, C. G.	Graduate Student, completed her Ph.D. degree
Barron, B. C.	Graduate Student, completed his Ph.D. degree
DeNagel, D. C.	Graduate Student, completed her Ph.D. degree
Slingsby, C.	Research Collaborator / Birkbeck College of Univ. of London
Bando, M.	Research Associate

Publications resulting from this funded project:

1. Sebag, J., Nie, S., Reiser, K., Charles, M. A., and Yu, N.-T. **(1994)** "Raman Spectroscopy of Human Vitreous in Proliferative Diabetic Retinopathy" **Invest. Ophthalmol. Vis. Sci.**, *35*, 2976-2980.

2. Lo, W.-K., Kuck, J. F. R., Shaw, A. P. and Yu, N.-T. **(1993)** "The Altricial Pigeon is Born Blind with A Transient Glycogen Catartact " **Exp. Eye Res.** *56*, 121-126.

3. Castillo, C. G., Lo, W.-K., Kuck, J. F. R., Jr. and Yu, N.-T.**(1992)** "Nature and Localization of Avian Lens Glycogen by Electron Microscopy and Raman Spectroscopy" **Biophys. J.** *61*, 839-844.

4. Bergbauer, K. L., Kuck, John F. R., Jr. Su, K C. and Yu, N.-T. **(1991)** "Use of a UV-Blocking Contact Lens in Evaluation of UV-Induced Damage to the Guinea Pig Lens" **Int. Contact Lens Clinic.** *18*, 182-187.

5. Zigman, S., Paxhia, T., McDaniel, T., Lou, M. F. and Yu, N.-T. **(1991)** "Effect of Chronic Near-Ultraviolet Radiation on the Gray Squirrel Lens *in Vivo*" **Invest. Ophthalmol. Vis Sci.**, *32*, 1723-1732.

6. Chen, Wenlung, Nie, S., Kuck, J.F.R., Jr., and Yu, N.-T. **(1991)** "Near-IR Fourier Transform Raman and Conventional Raman Studies of Calf g-Crystallins in the Lyophilized State and in Solution" **Biophys. J.** *60*, 447-455.

7. Yu, N.-T., Cai, M.-Z., Lee, B.-S., Kuck, J.F.R., Jr., McFall-Ngai, M. and Horwitz, J. **(1991)** "Resonance Raman Detection of a Carotenoid in the Lens of the Deep-Sea Hatchetfish" **Exp. Eye Res.** *52*, 475-479.

8. Nie, S., Bergbauer, K. ,L., Kuck, J.F.R., Jr., and Yu, N.-T. **(1990)** "Near Infrared Fourier Transform Raman Spectroscopy in Human Lens Research" **Exp. Eye. Res.** *51*, 619-623.

9. Yu, N.-T. and Nie, S. **(1990)** "Surface-Enhanced Hyper-Raman and Near-IR FT-Raman Studies of Biomolecules" **SPIE**, Vol. 1403, pp. 112-120, **Laser Applications in Life Sciences.**

10. Bursell, S.-E. and Yu, N.-T. **(1990)** "Fluorescence and Raman Spectroscopy of the Crystallin Lens" *in* Noninvasive Diagnostic Techniques in Ophthalmology (Masters, B., Ed.), pp. 319-341, Springer-Verlag New York Inc., New York, NY.

11. Zigman, S., Paxhia, T., Lou, M. and Yu, N.-T. **(1990)** "Comparative Study of Lens Proteins of Gray Squirrel and Human" **Com. Biochem. Physiol.** *96B*, 697-704.

12. Nie, S., Bergbauer, K. L., Ho, J. J., Kuck, J.F.R., Jr. and Yu, N.-T. **(1990)** "Applications of Near-Infrared Fourier Transform Raman Spectroscopy in Biology and Medicine" **Spectroscopy** *5*, 24-32.

13. Nie, S., Castillo, C. G., Kuck, J.F.R., Jr., Nabiev, I. and Yu, N.-T. **(1990)** "Surface-Enhanced Raman Spectra of Eye Lens Pigments" **Applied Spectrosc.** *44*, 571-575.

14. Yu, N.-T., Bando, M. and Kuck, J.F.R., Jr. **(1990)** "Localization of UV-Induced Changes in Mouse Lens" **Exp. Eye Res.** *50*, 327-329.

15. Yu, N.-T., Barron, B. C. and Kuck, J.F. R., Jr. **(1989)** "Distribution of Two Metabolically Related Fluorophors in Human Lens Measured by Laser Microprobe" **Exp. Eye Res.**, *49*, 189-194.

16. Cai, M.-Z., Kuck, J.F.R., Jr. and Yu, N.-T. **(1989)** "Galactose-Induced Cataract in Rat: Raman Detection of Sulphydryl Decrease and Water Increase Along an Equatorial Diameter" **Exp. Eye Res.** *49*, 531-541.

17. Yu, N.-T., DeNagel, D. C. and Slingsby, C. **(1989)** " Raman Spectroscopy of Calf Lens g-II Crystallin: Direct Evidence for the Formation of Mixed Disulfide Bonds with 2-Mercaptoethanol and Glutathione" **Exp. Eye Res.** *48*, 399-410.

18. Barron, B. C., Yu, N.-T. and Kuck, J.F.R., Jr. **(1988)** "Distribution of a 488.0-nm-excited Fluorophor in the Equatorial Plane of the Human Lens by a Laser

Raman Microprobe: A New Concept in Fluorescence Studies" **Exp. Eye Res.** *47*, 901-904.

19. Yu, N.-T. and Bursell, S. E. **(1988)** "A New Approach to Study Human Cataractogenenesis: Fluorescence / Raman Intensity Ratio and Imaging" *in* Spectroscopic and Structural Studies of Biomaterials I: Proteins (Edited by J. Twardowski) Sigma Press, Wilmslow, Cheshire, U. K. pp.65-76.

20. DeNagel, D. C., Bando, M., Yu, N.-T. and Kuck, J.F.R., Jr. **(1988)** "A Raman Study of Disulfide and Sulfhydryl in the Emory Mouse Cataract" **Invest. Ophthalmol. Vis. Sci.** *29*, 823-826.

21. Barron, B. C., Yu, N.-T. and Kuck, J.F.R., Jr. **(1988)** "Raman Spectroscopic Evaluation of Aging and Long-Wave UV Exposure in the Guinea Pig Lens: A Possible Model for Human Aging" **Exp. Eye Res.** *46*, 249-258.

22. Yu, N.-T., Cai, M.-Z., Ho, D. J.-Y. and Kuck, J.F.R., Jr. **(1988)** "Automated Laser Scanning Microbeam Fluorescence / Raman Image Analysis of Human Eye Lens with Multichannel Detection: Evidence for Metabolic Production of a Green Fluorophor" **Proc. Natl. Acad. Sci. USA,** *85*, 103-106.

Significant findings may be summarized as follows:

(a) We have examined the efficacy of a UV-absorbing contact lens in reducing UV-induced damage to the guinea pig lens *in vivo*. Each of the animals was fitted with a UV-blocking contact lens in one eye and a regular contact lens in the opposite eye. After continuous exposure to UV irradiation for several months, the two eye lenses from each animal were examined for any differences in appearance, disulfide concentration, and fluorescence. The study demonstrated that some of the same kinds of changes that occur during human aging and cataract formation (e.g., increased pigmentation, fluorescence, and disulfide cross-linking) may be produced in the guinea pig lens by UV irradiation at levels that approximate natural sunlight. Furthermore, this damage seems to occur by direct interaction of UV with the eye because the lenses of animals that received UV exposure while wearing a UV-blocking contact lens were not significantly affected.

(b) NIR FT-Raman Study of Tryptophan Photolysis: Photochemical lens damage involving the aromatic residues of lens proteins, particularly tryptophan residues, have been previously studied in the literature. It was reported that tryptophan residues were the major photochemically active absorbers in the near-UV-induced lenticular photodamage, and that N-formylkynurenine was its primary photoproduct. Currently, it is believed that the formation of N-formylkynurenine occurs by the opening of the indole ring of tryptophan but, to date, direct proof of this event has not been made available in the literature. We have sought to study the process of tryptophan photodestruction and the formation of its photoproducts, by NIR FT-Raman spectroscopy. The finger-printing capabilities of Raman spectroscopy, combined with the fluorescence rejection and multiplex / throughput advantages afforded by using near-IR excitation and Fourier-transform instrumentation, make NIR FT-Raman an ideal choice for studying such a process. We have monitored the decrease in the intensity of the 756 cm-1 Raman line of tryptophan, purported to be tryptophan's indole ring-breathing vibration, versus the intensity of the 984 cm-1 Raman line of sulfate (used as a standard). We have also studied the "quenching" effect on tryptophan fluorescence by its photoproducts which, albeit small, render a 20% error in the fluorescence intensity measurements.

(unpublished results from Ph.D. thesis by Dr. Carolina G. Castillo, June 1993)

(c) Nature and Localization of Avian Lens Glycogen by Electron Microscopy and Raman Spectroscopy : Glycogen was found to locate primarily in the nucleus of the lens (along the visual axis) and away from the metabolically active cortex suggesting a structural function. TEM showed that the glycogen particles were of the beta type (15-40 nm dia.) which are normally found in tissues with high metabolic activity (i.e., musculature, spermatozoa); however, larger glycogen particles such as the alpha particle (60-2000 nm diam.), known to play a structural role, would be of a size which could scatter light and its existence in the transparent lens could not be tolerated. The mass of evidence, then, indicated that glycogen in the lens nucleus of flying birds played a structural role in maintaining the refractive index of the lens.

(d) Distribution of two Metabolically Related Fluorophors in Human Lens : We employed the automated scanning Raman microprobe developed in our lab. to obtain the distribution of a major fluorophor, 3-OH-L-kynurenine-O-β-glucoside, in humen lenses from 0.38 to 71 yr. A three-dimensional perspective grid map with fluorescence intensity as the third dimension shows maximum fluorescence in the infant lens nucleus. At 12 yr the fluorescence peak is broadened and a toroid-shaped maximum occurs also in the outer cortex, creating a toroid-shaped minimum between the two maxima. By 71 yr the nuclear maximum is lower but a new (green) fluorophor (excitation 488 nm; emission 530 nm) has appeared as a toroidal maximum in the same location as the blue minimum, suggesting the conversion of the blue fluorophor to the unidentified green fluorophor.

Clinical Article

Use of a UV-Blocking Contact Lens in Evaluation of UV-Induced Damage to the Guinea Pig Lens

Katrina L. Bergbauer, John F.R. Kuck, Jr., PhD, Kai C. Su, PhD, and Nai-Teng Yu, PhD

Much evidence has been accumulated to suggest that UV-A and UV-B of sunlight, which penetrate the cornea and are partly absorbed by the lens, are important factors in cataractogenesis. We have examined the efficacy of a UV-absorbing contact lens in reducing UV-induced damage to the guinea pig lens in vivo. *Each of the animals was fitted with a UV-blocking hydrogel contact lens, possessing a monolayer of UV-absorbing chromophore, on the control eye and a regular hydrogel contact lens on the contralateral eye. After 12–19 months of continuous exposure to UV from a blacklight source, the unprotected lens showed increased (a) opacification, (b) pigmentation, (c) fluorescence, and (d) disulfide formation. Such changes also occur in human lenses during cataractogenesis. Therefore, this study strongly supports the idea that increased exposure to UV light is an important causative factor in human cataract formation.*

Keywords: Hydrogel contact lenses; UV blocking; cataracts

Introduction

The detrimental effects of UV exposure on human health have attracted much attention in recent years. Most humans receive UV exposure, primarily from sunlight, throughout their lifetimes, and the link between sun exposure (i.e., UV-B) and skin damage resulting in premature aging and, in some cases, skin cancer is well recognized.[1] In addition, there are indications that the eye lens is also susceptible to UV insult. Epidemiologic studies have shown a positive correlation between sun exposure and cataracts,[2,3] and UV irradiation of animal lenses *in vitro* and *in vivo* has produced a number of lens alterations (e.g., opacification, increased coloration and fluorescence, increased cross-linking) that are reminiscent of human lens aging and cataract formation.[4–6] Still, the aforementioned studies do not constitute final proof per se of a connection between UV and cataracts, and more studies are needed for unequivocal determination that such a relationship exists.

When the results produced from UV studies of animal lenses are to be interpreted in terms of human cataract formation, it is important to justify insofar as possible the choice of animal. In one study that compared human lenses with the lenses of several types of animals often utilized in UV/lens experiments, the amount of disulfide formed during normal aging was determined.[7] Disulfide bonds (represented by S–S) are formed when two sulfhydryl groups (–SH) in the lens protein interact; so disulfide concentrations represent one type of lens cross-linking. These studies showed that human and guinea pig lenses develop very few disulfide cross-links during normal aging, but in other rodents such as mice and rats, sulfhydryl-to-disulfide conversion is a normal aging process. Also, concomitant with this sulfhydryl conversion, mouse and rat lenses develop hard nuclei, while human and guinea pig lenses do not display this characteristic. Thus, it appears that, among the ani-

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Address reprint requests to Dr. Su at the Advanced Technology Division, Ciba Vision Care, 5000 McGinnis Ferry Rd., Alpharetta, GA 30201, USA.

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mals commonly used for laboratory research, the guinea pig lens more closely resembles the human lens than do the lenses of other species. In a recent comparison of lens disulfide concentrations in UV-irradiated and normal control guinea pigs, UV radiation was shown to accelerate the formation of disulfide cross-links.[4] Interestingly, slit-lamp examinations performed prior to disulfide measurement showed that a slight nuclear haze was present in the lenses of the UV-exposed animals. The authors therefore concluded that the UV-induced disulfide cross-links in the guinea pig lenses might be precataractous. Other investigators have suggested that disulfide formation may be important in the pathogenesis of human cataracts as well because human cataracts are enriched in this type of cross-linking as compared to normal lenses.[8,9] However, since the guinea pig studies utilized different animals for their control and irradiated lenses, the possibility that the observed lens changes may have been initiated systemically cannot be ruled out. To determine whether UV irradiation is directly damaging to the eye lens, we have evaluated the effect of UV radiation on the guinea pig lens. Unlike previous such studies, our control and UV-irradiated lenses are from the same animal. We accomplished this by fitting each guinea pig with a UV-blocking contact lens in one eye and a regular contact lens on the opposite eye. After UV irradiation for several months, the two eye lenses from each animal were examined for any differences in appearance, disulfide concentration, and fluorescence. By this experimental approach, any observed differences between lenses would unarguably be a result of a direct photochemical process in the lens. In addition, we also preexposed some of the guinea pigs to UV for 4 months prior to initiating lens wear so that we could evaluate the cumulative nature of UV exposure and determine whether UV-associated lens damage could be suspended once initiated.

Materials and Methods

Guinea pigs were fitted with regular and UV-absorbing hydrogel contact lenses on their right and left eyes, respectively, and exposed to long-wave UV continuously for up to 19 months. In one group of animals, contact lens wear and irradiation were initiated simultaneously at 5 months of age; in another group, contact lens wear was not started until the subjects had received 4 months UV exposure. The regular contact lenses were tefilcon (Cibasoft, Ciba Vision Corp.) and UV-absorbing tefilcon (Ciba Vision Corp.). Contact lenses were removed each week and replaced with new lenses.

UV irradiation was provided by a General Electric UV lamp (F-15T8 BLB, Cleveland, OH) with an output of 4 mW/cm^2 at the lamp surface over a 305–410 nm spectral region and peak emission at 365 nm. The range of the ultraviolet spectrum supplied by this lamp is very similar to that present in natural sunlight in that it contains a portion of the UV-B band (normally defined as 290–320 nm) and also encompasses all of the UV-A band containing wavelengths from 320 to 400 nm. As UV-C (100–290 nm) is not normally encountered in our natural environment and is present only in specific industrial situations, it was not necessary to consider its effect in this particular study. During the irradiation period, the animals displayed no adverse symptoms (such as hair loss or skin lesions) except for slight drying of the outer ear. The UV transmittance spectra of the regular and UV-absorbing contact lenses are shown in *Figure 1*, demonstrating that the regular contact lens transmits all of the radiation emitted by the UV lamp while the UV-absorbing lens blocks most of the lamp's output.

Housing conditions for normal and UV-irradiated guinea pigs were as previously described.[5] The ocular lenses were dissected from the enucleated eyes of each guinea pig, and after visual inspection and photography, laser Raman spectroscopy was utilized for determination of disulfide and sulfhydryl concentrations along the lens visual axis. The Raman system and lens analysis procedures have been described elsewhere.[4] Briefly, the lenses were placed anterior side down in a quartz cuvette containing a 0.9% saline/glucose medium. The cuvette was placed on a precision X–Y translation stage equipped with two micrometers that allowed the lens to be oriented such that the laser beam traversed the visual axis (VA) from below without deflec-

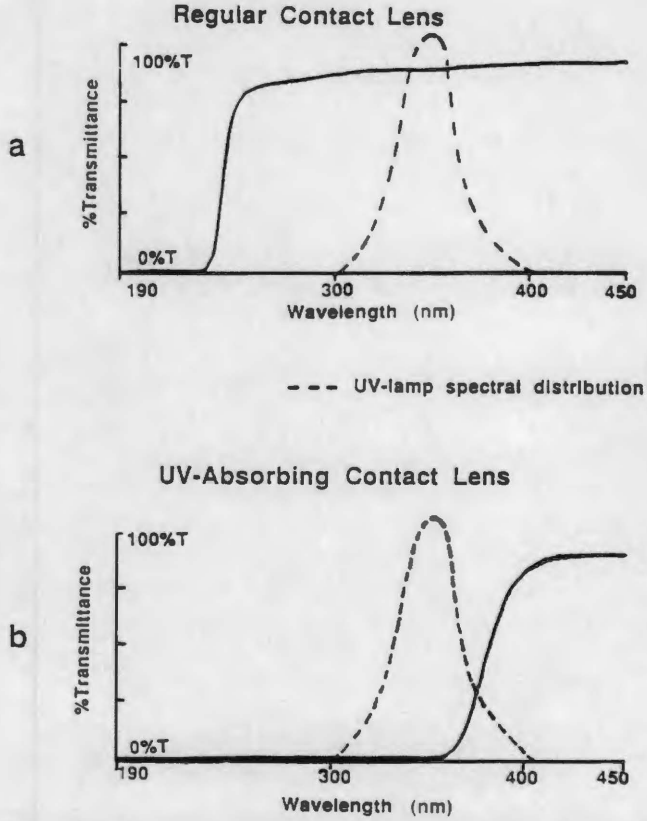

Figure 1. UV-transmittance spectra for a regular hydrogel contact lens (a) and a UV-absorbing (UV-blocking) contact lens (b). The UV-lamp output is represented only in terms of its spectral range; intensity is in arbitrary units.

tion. The laser image of the lens was focused on the entrance slit of a Spex 1401 double monochromator, and the VA length was determined accurately from the lens image. The VA was then divided into 11 or 21 equidistant points, and a spectrum was obtained for each point by analyzing the Raman scattered light 90° from the incident laser beam. Sulfhydryl profiles were constructed by standardizing the intensity of the sulfhydryl signal at 2580 cm^{-1} to the intensity of the protein reference signal (2731 cm^{-1}) for each point analyzed along the VA. The intensity ratio for each point was then plotted against the distance from the nuclear center of the lens and curve fit using a third-order polynomial equation. Disulfide profiles were generated similarly using the intensity ratio of disulfide (508 cm^{-1}) to the phenylalanine signal at 622 cm^{-1}. Using laser excitations 457.9 and 514.5 nm, we also used the Raman system for fluorescence measurements. Fluorescence profiles were constructed by standardizing the fluorescence intensity (I_F) by the Raman protein signal (I_{RP}) at 2940 cm^{-1}, and this fluorescence ratio was plotted against the distance from the lens nuclear center.

Results

Sulfhydryl and Disulfide Analyses

Figure 2a and *b* shows the lens sulfhydryl profile for guinea pigs receiving 12 and 19 months UV-irradiation *in vivo*, respectively. Each of these animals wore a UV-blocking contact lens on its left eye and a regular contact lens on its right eye. Comparison of the sulfhydryl profiles for the left and right lenses indicates that after 12 months UV-irradiation, the nonprotected (right) lens had approximately 20% less sulfhydryl at the nuclear center relative to the UV-protected (left) lens. Similarly, in the guinea pig that was irradiated for 19 months, the sulfhydryl concentration was 40% lower in the right lens as compared to the left lens. As shown in *Figure 2c*, the sulfhydryl concentrations within the left and right lenses from a normal, nonirradiated guinea pig are virtually identical, thus indicating that the left/right lens differences depicted in *Figure 2a* and *b* are significant and are not simply a result of normal variation between contralateral lenses within individual guinea pigs. *Figure 3* shows the disulfide profiles for a

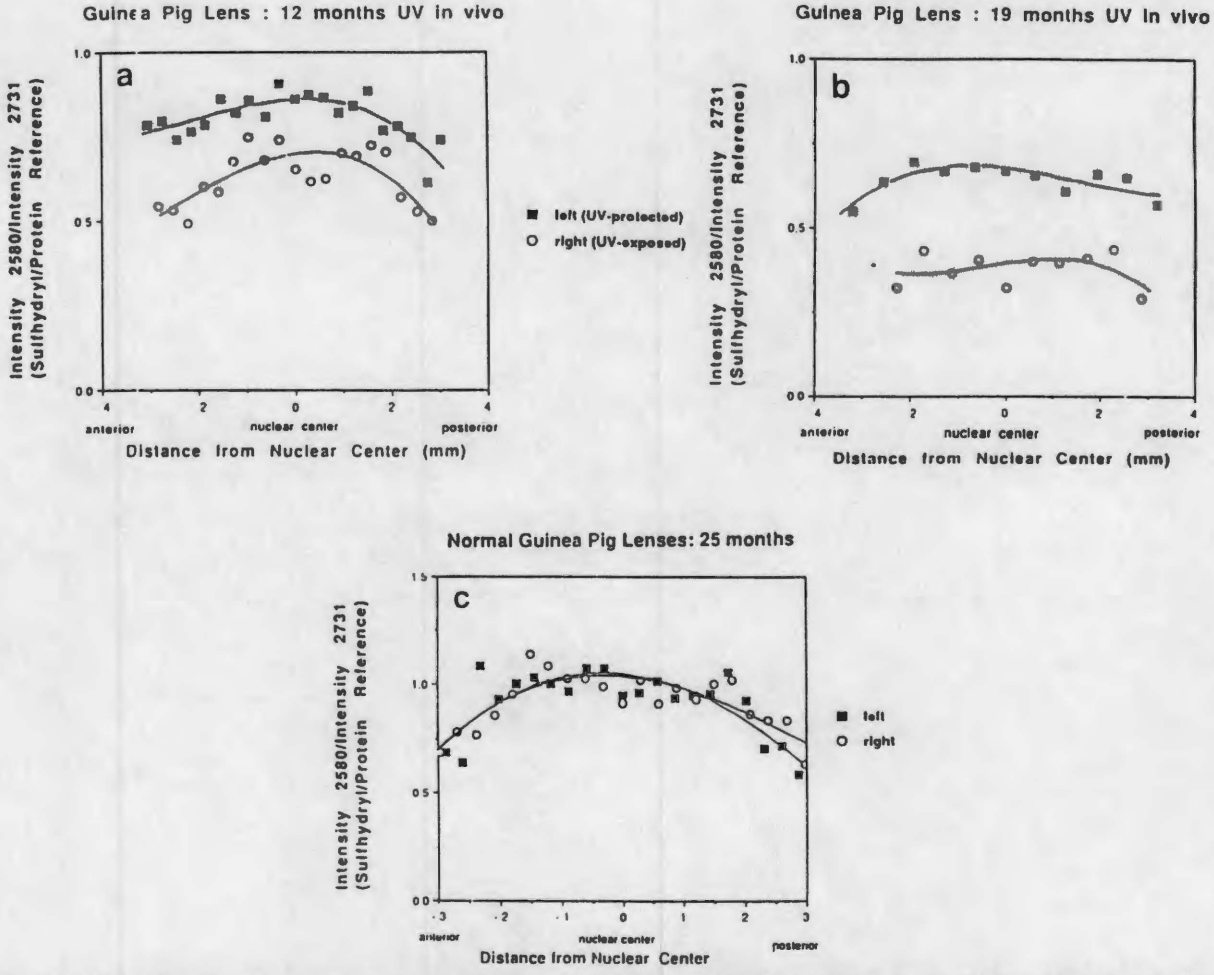

Figure 2. VA sulfhydryl profiles for a pair of guinea pig lenses after (a) 12 months UV irradiation *in vivo* (17 months old); (b) 19 months UV *in vivo* (24 months old); (c) 0 months UV (25 months old). Point #1 for the UV-exposed lens in *Figure 4b* is not included here due to an abnormally high level of –SH at the anterior capsule lens interface.

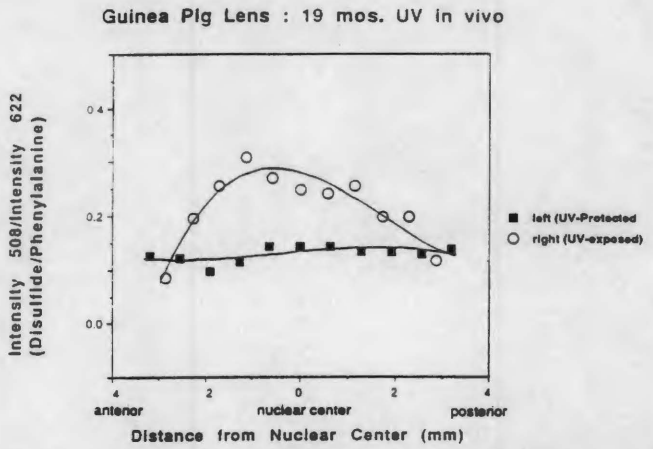

Figure 3. VA disulfide profile for a pair of 25-month-old guinea pig lenses after UV irradiation *in vivo* for 19 months. Left lens was UV-protected and right lens was UV-exposed.

pair of lenses from a guinea pig that was subjected to 19 months UV irradiation. These data were gathered from the same lenses whose sulfhydryl profiles are represented in *Figure 2b*. As expected, the decrease in sulfhydryl concentration for the UV-exposed (right) lens is accompanied by an increase (approximately 100%) in disulfide when compared to the UV-protected (left) lens.

Fluorescence Analysis

After 15 months UV irradiation of one guinea pig, extreme differences were noted between the left and right lenses. The right (nonprotected) lens was very yellow, yet the left (UV-protected) lens appeared to be relatively normal, possessing little coloration (*Figure 4a*). In addition, *Figure 4b* shows that the right lens was slightly cloudy as compared to the left lens. Examination of the fluorescence associated with these lenses (ex./em. 514.5/580.4) revealed that the UV-exposed (right) lens exhibited intense fluorescence (*Figure 5a*), while the UV-protected (left) lens was only slightly fluorescent (*Figure 5b*). In previous studies of UV-exposed guinea pig lenses, Barron *et al.*[5] detected another fluorophor (ex./em. 457.9 nm/497 nm); therefore, we also determined the amount of this fluorophor in our UV-exposed and UV-protected lens pairs. *Figure 6a* and *b* compares the geometric distribution of the 457.9 nm excited fluorophor of a UV-protected (left) and a UV-exposed (right) lens from a guinea pig after 15 months UV-irradiation. During the first 4 months of UV exposure, this particular animal did not wear any contact lenses so that we could provide initial UV insult to left and right eye lenses equally. The reason for this protocol variation was to allow us to determine whether the UV-blocking contact lens is effective in stopping the progression of UV-associated lens changes. *Figure 6c* shows that there is essentially no fluorescence present in the lens of a normal 21-month-old guinea pig. Altogether, *Figure 6* shows that fluorescence is elevated significantly at the nuclear center of the lens that received no UV protection during UV irradiation as compared to the lens that was protected by UV-blocking contact lens during the last 11 months of UV exposure.

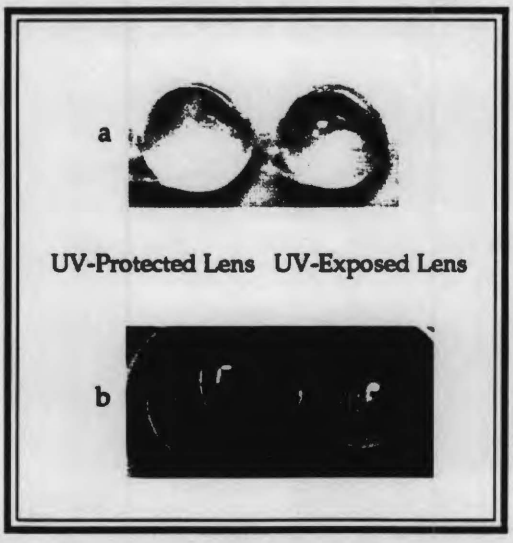

Figure 4. Photographs of the eye lenses from a guinea pig after 15 months UV irradiation *in vivo* comparing (a) lens color and (b) lens clarity. This animal wore a UV-blocking contact lens on its left eye and a regular contact lens on its right eye during the irradiation period.

Discussion

This study demonstrates that the UV-blocking contact lens reduces UV-associated damage to the guinea pig lens. Specifically, we showed that some of the same kinds of changes that occur during human lens aging and cataract formation (e.g., increased pigmentation, fluorescence, and disulfide cross-linking) can be produced in the guinea pig lens by UV irradiation at levels that approximate natural sunlight. Furthermore, this damage does seem to occur by

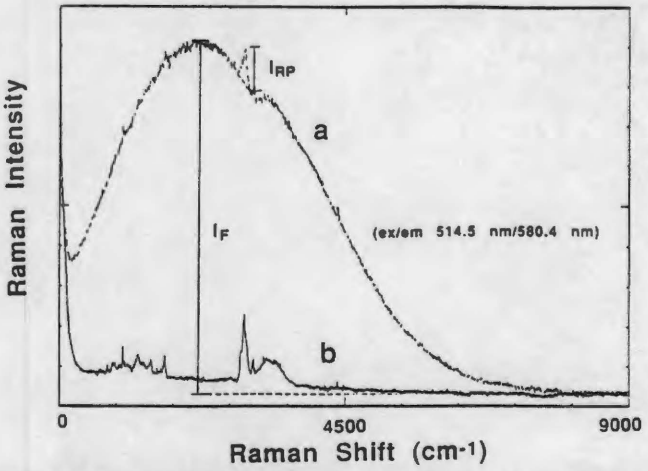

Figure 5. Fluorescence and Raman spectra for the (a) right (UV-exposed) and (b) left (UV-protected) lenses from a guinea pig after 15 months long-wave UV irradiation *in vivo*. Fluorescence excitation/emission = 514.5 nm/580.4 nm. Spectrum from lens nuclear center.

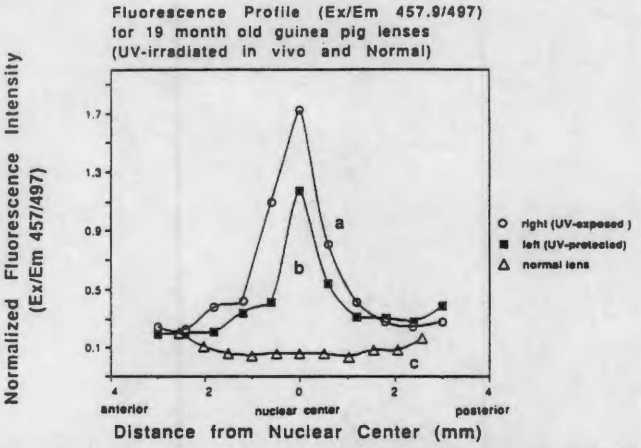

Figure 6. VA fluorescence (457.9 nm/497 nm ex./em.) profile for (a) UV-exposed and (b) UV-protected guinea pig lens after 15 months UV irradiation (11 months wearing contact lens) and (c) normal 19-month-old guinea pig lens.

direct interaction of UV with the eye because the lenses of animals that received UV exposure while wearing a UV-blocking contact lens were not significantly affected. This study also shows that UV damage to the guinea pig lens is dose-dependent since the magnitude of UV-induced lenticular fluorescence could be reduced by fitting preexposed animals with UV-blocking contact lenses. In light of the known similarities that exist between human and guinea pig lenses, the present study reinforces earlier work suggesting that the human lens also may be susceptible to UV radiation from the sun. It therefore also seems prudent to address the efficacy of products aimed at counteracting UV effects on the eye lens. In this respect, the results of this study suggest that the UV-blocking contact lens may be particularly useful for future protection of the human lens from the damaging effects of UV radiation.

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Katrina L. Bergbauer is pursuing her PhD in biochemistry at Georgia Institute of Technology. She is also an associate research scientist at the Advanced Technology Division of CIBA Vision Corporation. Her current research interests lie primarily in the area of Raman spectroscopic characterization of eye lens aging and cataract formation.

Dr. John F.R. Kuck earned his BS and MS in chemistry from the Virginia Polytechnic Institute and his PhD in biochemistry from the University of North Carolina. He joined the Kresge Eye Institute in Detroit, Michigan, in 1957 and moved to Emory University in 1963 where he became emeritus professor of ophthalmology in 1988. He is currently visiting professor of chemistry and biochemistry at the Georgia Institute of Technology. Most of his career has been devoted to the study of lens metabolism and cataract formation.

Dr. Kai C. Su, executive vice president, CIBA Vision Corporation, directs the activities and is head of CIBA Vision's Advanced Technology Division. He is the inventor of the SOFTCOLORS™ and Illusions tint processes as well as many advancements in lens quality and production technology. A native of Hupei, China, Dr. Su attended college in Taiwan and received his PhD in polymer chemistry at Polytechnic Institute, Brooklyn, New York, in 1971.

Dr. Nai-Teng Yu has been professor of chemistry at Georgia Institute of Technology since 1970. In July 1990, Dr. Yu took a sabbattical leave from the Georgia Institute of Technology to become a founding head of the Department of Chemistry at the Hong Kong University of Science and Technology. Dr. Yu received his BS in 1963 in chemical engineering at National Taiwan University and a PhD in 1969 in biophysical chemistry at Massachusetts Institute of Technology. He has been a pioneer in the application of laser Raman spectroscopy to biological molecules, with special interest in noninvasive Raman and fluorescence imaging of the eye lens.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES

FINAL INVENTION STATEMENT AND CERTIFICATION

(FOR GRANT OR AWARD)

DHHS GRANT OR AWARD NO.
VISA-1
5 R01 EY01746-17

A. We hereby certify that, to the best of our knowledge and belief, all inventions are listed below which were conceived and/or first actually reduced to practice during the course of work under the above-referenced DHHS grant or award for the period

05/01/79 (original effective date) through 04/30/94 (date of termination).

B. INVENTIONS (Note: If no inventions have been made under the grant or award, insert the word "NONE" under Title below.)

NAME OF INVENTOR	TITLE OF INVENTION	DATE REPORTED TO DHHS
amuels, M.A., Patterson, S.W. pstein, J.A., Yu, N.-T. and ussell, S.E.	Apparatus and Methods for Quantitatively Measuring Molecular Changes in the Ocular Lens	
	U.S. Patent No. 5,203,328 (1993)	

e continuation sheet if necessary)

C. FIRST SIGNATURE — The person responsible for the grant or award is required to sign (in ink). Sign in the block opposite the applicable type of grant or award.

TYPE OF GRANT OR AWARD	WHO MUST SIGN (title)	SIGNATURE
search Grant	Principal Investigator ~~or Project Director~~	
alth Services Grant	Director	
search Career Program Award	Awardee	
other types *(specify)*	Responsible Official	

D. SECOND SIGNATURE — This block must be signed by an official authorized to sign on behalf of the institution.

.E	NAME AND MAILING ADDRESS OF INSTITUTION
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568 (Rev. 9/91)